

THE RELATIONSHIP OF EPINEPHRINE AND GLUCAGON TO LIVER PHOSPHORYLASE

IV. EFFECT OF EPINEPHRINE AND GLUCAGON ON THE REACTIVATION OF PHOSPHORYLASE IN LIVER HOMOGENATES*

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The concentration of active phosphorylase in liver represents a balance between inactivation by liver phosphorylase phosphatase (inactivating enzyme) and reactivation by dephosphophosphorylase kinase. The enzymatic inactivation of phosphorylase proceeds with the release of inorganic phosphate (2, 3), while the reactivation of dephosphophosphorylase requires magnesium ions and ATP¹ and proceeds with the transfer of phosphate to the enzyme protein (4).

It has been shown in liver slices that epinephrine and glucagon displace this balance in favor of the active phosphorylase (5, 6). This report is concerned with the demonstration of a similar effect in cell-free liver homogenates; *i.e.*, an increased formation of active phosphorylase occurred in cell-free homogenates in the presence of sympathomimetic amines and glucagon. The relative activities of the sympathomimetic amines in homogenates were found to be similar to the relative activities determined by liver slice technique or by injection into intact animals.

It has been possible to show that the response of the homogenates to the hormones occurred in two stages. In the first stage, a particulate fraction of homogenates produced a heat-stable factor in the presence of the hormones; in the second stage, this factor stimulated the formation of liver phosphorylase in supernatant fractions of homogenates in which the hormones themselves were inactive.

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¹ The following abbreviations are used: ATP, adenosine triphosphate; ADP, adenosine diphosphate; 5-AMP, adenosine-5-phosphate; Tris, tris(hydroxymethyl)aminomethane; TCA, trichloroacetic acid; LP, liver phosphorylase; dephospho-LP, liver dephosphophosphorylase; phosphokinase, dephosphophosphorylase kinase.

Methods

Preparation of Liver Homogenates—Mature dogs were killed by severing the arteries in the neck under deep secobarbital anesthesia. Mature cats were similarly killed under chloroform anesthesia. The livers were perfused with 0.9 per cent NaCl and sliced as previously described (4). The slices were rinsed with 5 volumes of 0.9 per cent NaCl and were shaken in air at 37° for 15 minutes in 2 to 3 volumes of a mixture containing 0.12 M NaCl plus 0.04 M glycylglycine buffer plus 0.001 M potassium phosphate buffer at pH 7.4. At the end of the incubation, the medium was decanted and the slices were rinsed twice with 3 to 4 volumes of cold 0.33 M sucrose. The slices (in 15 to 20 gm. portions) were then homogenized in 2 volumes of 0.33 M sucrose in an all-glass homogenizer. The homogenates were routinely centrifuged at $900 \times g$ for 1 minute before use.

Fractionation of Homogenates—Low speed centrifugations (up to $1200 \times g$) were conducted in a cold room at 3°, with the horizontal yoke (head No. 240) on the International centrifuge No. 2. Approximately 25 ml. portions of homogenate were placed in 45 ml. Lusteroid tubes and centrifuged for 10 minutes at the specified centrifugal force. The supernatant fluid ($1200 \times g$ supernatant fraction) was removed by aspiration. The precipitate was rehomogenized in an equal volume of 0.25 M sucrose, and the suspension diluted to the original volume of the homogenate. For experiments in recombination, these suspensions were centrifuged in 25 ml. portions at successively higher speeds, and the resulting precipitate fractions were suspended in the $1200 \times g$ supernatant fraction. For other experiments, these suspensions were centrifuged at $1200 \times g$, and the resulting precipitate was suspended in an equal volume of 0.25 M sucrose (washed liver particles).

The $11,000 \times g$ supernatant fraction was prepared by centrifugation of either the $1200 \times g$ supernatant fraction or the whole homogenate for 15 minutes at $11,000 \times g$ on the Spinco preparative ultracentrifuge; for some experiments, this fraction was centrifuged at either $50,000 \times g$ for 1 hour or $100,000 \times g$ for 45 minutes to remove the formed elements. In all cases, the supernatant fluid was removed by aspiration. The $100,000 \times g$ supernatant fraction at times was dialyzed *versus* 150 volumes of distilled water for 3 hours with shaking.

Assay of LP in Homogenates and Fractions of Homogenates—Aliquots of a homogenate or fraction were added to iced culture tubes containing various additions, bringing the final volume to 0.20 to 0.25 ml. The basic phosphorylase assay reagent (2.8 ml.), containing glucose-1-phosphate, glycogen, and 5-AMP (7), was added either immediately or after 5 to 10 minutes of shaking at 30°. After addition of the assay reagent, the tubes were incubated 10 minutes at 37°, and the assay was terminated by

the addition of 1.0 ml. of 15 per cent TCA. The inorganic phosphate present in an equivalent of 0.15 ml. of reaction mixture was determined by the method of Fiske and Subbarow (8), as adapted to the Klett-Summerson photometer. Units of phosphorylase activity were calculated as defined previously (7).

Materials—Dephospho-LP was prepared from dog liver as described previously (4). Amorphous glucagon samples (about 50 per cent pure) were donated by Eli Lilly and Company. *l*(-)-Epinephrine bitartrate, *d*(+)-epinephrine, *l*(-)-arterenol bitartrate (*l*(-)-norepinephrine), and *d*(+)-arterenol bitartrate (*d*(+)-norepinephrine) were kindly supplied by M. L. Tainter. Amphetamine (Benzedrine) was obtained as the sulfate salt and ATP as the crystalline disodium salt. Tris was recrystallized before use (7).

Results

Effects of Epinephrine and Glucagon in Whole Homogenates—Aliquots of homogenates were incubated at 30° with buffer, magnesium ions, and ATP in the absence and in the presence of epinephrine or glucagon. Phosphorylase activity was assayed before and after a 10 minute incubation (Fig. 1, left-hand bars). Since the homogenate was derived from preincubated slices, the initial level of active LP was low, most of the phosphorylase being present as dephospho-LP. In the absence of the hormones, only a small amount of dephospho-LP was converted to LP during the incubation of the homogenate. However, in the presence of the hormones, the formation of LP was increased nearly 4-fold. When the homogenate was supplemented with purified dephospho-LP, the effect of the hormones was magnified so that the formation of LP in the presence of the hormones was nearly 7 times that in their absence (Fig. 1). The formation of LP in homogenates in either the absence or presence of the hormones required the addition of both ATP and magnesium ions.

Increased formation of LP in the presence of epinephrine and glucagon also occurred in homogenates which had been frozen and thawed (Fig. 2). Some preparations (dog liver homogenates) have been frozen and stored at the temperature of solid CO₂ for a few weeks without appreciable change in properties, except those ascribable to the initial freezing process. The principal effect of freezing or other methods of storage of homogenates was an increased formation of phosphorylase in the absence of the hormones, with only a small diminution of the formation of phosphorylase in their presence.

The assumption that an increase in the phosphorylase activity of a homogenate corresponded to an increase in the amount of LP formed was substantiated by an experiment in which the phosphorylase activity of

homogenates incubated with and without epinephrine or glucagon was assayed before and after precipitation with ammonium sulfate. The increase in phosphorylase activity after incubation with the hormones was still present after the protein was precipitated twice at 0.67 saturation with ammonium sulfate.

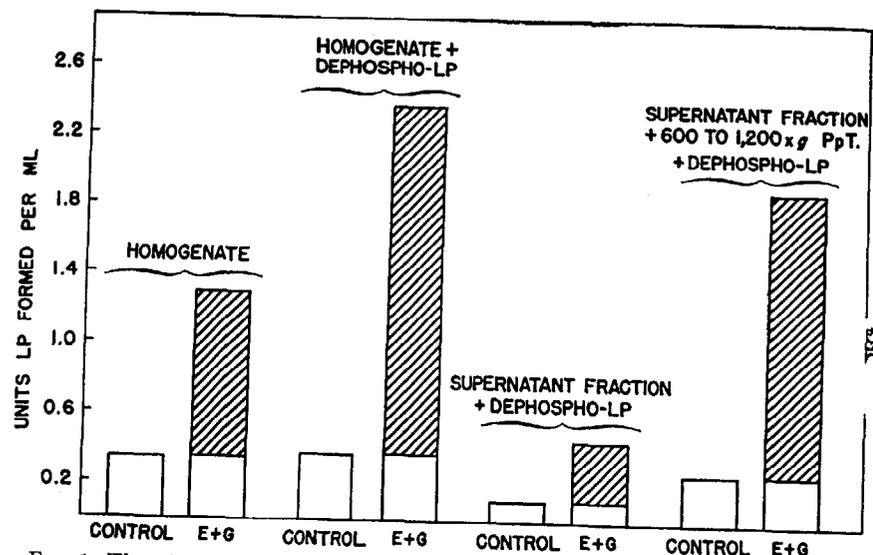


FIG. 1. The effect of epinephrine and glucagon on LP formation in a whole and fractionated cat liver homogenate. 0.14 ml. of homogenate or homogenate fraction was incubated with 2×10^{-3} M Tris buffer (pH 7.4), 2.5×10^{-3} M $MgSO_4$, and 1.7×10^{-3} M ATP in the presence and absence of 0.4γ of *l*-epinephrine plus 1.0γ of glucagon. The final volume was 0.20 ml. Dephospho-LP (4.2 units per ml.) was added where indicated. The supernatant fraction used in this experiment was the $1200 \times g$ supernatant fraction. LP activity was assayed before and after 10 minutes incubation at 30° . The bars represent the amount of LP formed during the incubation period; the cross-hatched portions of the bars represent the increased LP formation above that of the control.

Participation of Particulate Fractions Other Than Intact Cells in Response of Liver Homogenates—The probability that the response to epinephrine and glucagon in liver homogenates was restricted to unbroken cells remaining in the homogenates was small because, first, partially purified dephospho-LP added to liver homogenates participated in the response to the hormones (Fig. 1), and, second, the response to the hormones in homogenates survived the process of freezing (Fig. 2). Furthermore, it was possible to observe a good hormone response in preparations which contained no microscopically detectable intact cells. The preparation used in the experiment of Fig. 1 (right-hand bars) was composed of a washed

particulate fraction collected at 600 to $1200 \times g$ and the $1200 \times g$ supernatant fraction. Microscopic examination of this preparation, with use of Wright's stain or Leishman's stain, did not reveal the presence of either intact cells or intact nuclei.

Centrifugation of homogenates at $1200 \times g$ or more virtually abolished the hormone response in the resultant supernatant fraction (Figs. 1 and 3).

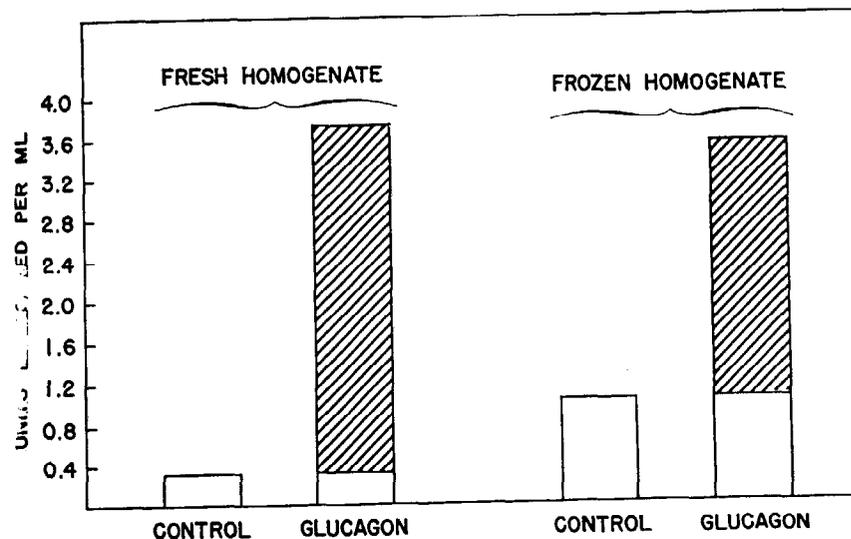


FIG. 2. The effect of glucagon on LP formation in a fresh and a frozen homogenate of dog liver. 0.15 ml. of homogenate was incubated for 10 minutes at 30° with 4×10^{-2} M Tris buffer (pH 7.4), 4×10^{-3} M $MgSO_4$, 2.8×10^{-3} M ATP, 4.2 units per ml. of dephospho-LP, and 0.1 mg. per ml. of casein in the presence and absence of 2.5γ of glucagon. The final volume was 0.25 ml. The experiment depicted in the left-hand bars was performed immediately after preparation of the homogenate, and the experiment depicted in the right-hand bars with an aliquot of the homogenate which had been frozen in a dry ice-alcohol bath and stored 24 hours in solid CO_2 .

The recombination of any portion of the particulate fractions sedimenting at $1200 \times g$ or less with supernatant fractions resulted in preparations which responded to the hormones. In the example shown in Fig. 1 (right-hand portion), the addition of a small amount of a washed particulate fraction collected at 600 to $1200 \times g$ to a $1200 \times g$ supernatant fraction restored to a large extent the response to the hormones observed in the whole homogenate. In this experiment, particulate fractions collected at 0 to $300 \times g$ and 300 to $600 \times g$ were equally effective in restoring the hormone response in the $1200 \times g$ supernatant fraction. In another experiment, a mixture of washed particles collected at $1200 \times g$ and the $11,000 \times g$

supernatant fraction exhibited the same hormone response as the homogenate from which the fractions were derived.

These experiments have not established any of the cell fractions obtainable by differential centrifugation as the locus of the particle primarily responsible for the response to the hormones. Intact cells and intact nuclei appear to be excluded by microscopic examination of active preparations. Furthermore, results to date have not indicated a close association of the active particles to the mitochondria. Supernatant fractions, prepared by

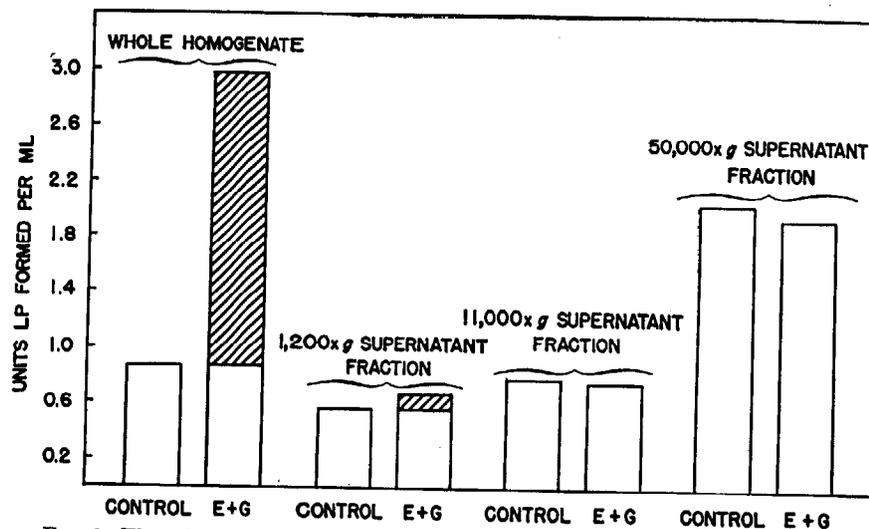


FIG. 3. The effect of epinephrine and glucagon on LP formation in fractions of a cat liver homogenate. 0.14 ml. of homogenate or homogenate fraction was incubated 10 minutes at 30° with 4×10^{-2} M Tris buffer (pH 7.4), 2.5×10^{-2} M $MgSO_4$, 1.7×10^{-3} M ATP, and 4.2 units per ml. of dephospho-LP in the presence and absence of 0.4 γ of *l*-epinephrine plus 2.0 γ of glucagon. The final volume was 0.20 ml.

centrifuging homogenates at $1200 \times g$, had little or no ability to respond to the hormones (Figs. 1 and 3); these fractions would be expected to contain the major portion of the mitochondria. However, cytochrome oxidase determinations by the method of Cooperstein and Lazarow (9) indicated that $1200 \times g$ supernatant fractions contained only about 30 to 35 per cent of the cytochrome oxidase activity of the whole homogenate. Since such a large proportion of the mitochondria appeared to be in the particulate fractions collected at $1200 \times g$, it is difficult to rule out the possibility that recombination procedures raised the ratio of mitochondria to other cell fractions above some critical level necessary for the hormone response. In any event, it is evident that preparations derived from liver homogenates required the presence of some particulate fraction in order to show a sig-

nificant increase in the formation of LP in the presence of epinephrine or glucagon.

Relative Activities of Sympathomimetic Amines and Glucagon in Liver Homogenates—The magnitude of the increased formation of phosphorylase in the presence of the hormones was related to the amount of epinephrine or glucagon added to the homogenates. The half maximal response occurred at a concentration below 1×10^{-8} M with glucagon and 1×10^{-7} M with *l*-epinephrine.² It was of interest to estimate the relative activities of compounds related to *l*-epinephrine in the liver homogenate system,

TABLE I

Relative Activities of Sympathomimetic Amines in Vitro and in Vivo

For the liver homogenate assay, 0.15 ml. of frozen dog liver homogenate, diluted 2.5-fold with 0.33 M sucrose after thawing, was incubated 10 minutes at 30° with 5×10^{-2} M Tris buffer (pH 7.4), 5×10^{-3} M $MgSO_4$, 3.5×10^{-3} M ATP, dephospho-LP (4.8 units per ml.), and bovine serum albumin (8 γ per ml.) in a final volume of 0.25 ml. The increase in LP formation owing to the addition of *l*-epinephrine (5.4×10^{-8} M to 3.2×10^{-7} M) was compared to that owing to the addition of various amounts of the other sympathomimetic amines listed below. The values express the potency of these compounds relative to that of *l*-epinephrine calculated on a molar basis.

Sympathomimetic amine	Relative activity		
	Liver homogenate assay	Liver slice assay*	Intact animal assay
<i>l</i> -Epinephrine	100	100	100†
<i>l</i> -Norepinephrine	10	16	12†
<i>d</i> -Epinephrine	12	16	
<i>d</i> -Norepinephrine	0.4	2	0.6†
Amphetamine	0.0006	0.0	0.0

* Calculated from the data of Sutherland and Cori (5).

† Determined by McChesney *et al.* (10).

since these compounds vary in potency *in vivo*. In Table I are listed the relative activities of *l*-epinephrine, *d*-epinephrine, *l*-norepinephrine, *d*-norepinephrine, and amphetamine in stimulating the net formation of LP in liver homogenates. Included in Table I for comparison are relative activities of these compounds in stimulating glucose output of liver slices and in causing hyperglycemia in the intact animal. It can be seen that the activities of the compounds relative to that of *l*-epinephrine in liver homogenates are similar to those observed in the other systems.

² The adaptation of the homogenate system to the measurement of small amounts of epinephrine and glucagon involved modification of the conditions recorded in Fig. 1. The details of these modifications, as well as some applications of this assay system, will be reported in a subsequent publication.

Production of Factor Active in LP Formation by Particulate Fractions of Homogenates in Presence of Hormones—The observation that particulate fractions of liver homogenates were essential for the effect of epinephrine and glucagon on LP formation prompted experiments in which the washed particulate material was incubated with the hormones. Fig. 4 depicts the results of a typical experiment. Aliquots of a suspension of washed liver particles, collected at $1200 \times g$, were incubated with ATP and magnesium ions in the absence and presence of a mixture of epinephrine and glucagon. The entire incubation mixtures were heated in boiling water, chilled, and centrifuged. Aliquots of the resulting supernatant fluid (referred to as "boiled extract" below) were incubated with ATP, magnesium ions, and an $11,000 \times g$ supernatant fraction. It can be seen from Fig. 4 that, in the presence of the boiled extract derived from particles incubated with the hormones, the formation of LP was increased and that the magnitude of this increase was related to the amount of boiled extract added to the incubation mixture. The boiled extract derived from particles incubated in the absence of the hormones, as well as a mixture of the hormones themselves, had only a small effect on LP formation. The addition of magnesium ions and ATP was found to be essential for production of the active principle in the presence of the hormones and liver particles and also for formation of LP in the $11,000 \times g$ supernatant fraction, either in the absence or presence of active preparations of the boiled extract.

Properties of Active Factor—The stimulation of LP formation in the $11,000 \times g$ supernatant fraction (Fig. 4) was used to estimate the amount of the unknown factor in crude or purified preparations. Before attempting purification procedures, some general information about the stability of the factor was gathered. The factor survived heating in boiling water for 3 minutes at pH 7.4 during preparation of the boiled extracts, as well as incubation for 24 hours at 25° in $0.1 N HCl$. After being heated for 30 minutes in boiling water in $0.05 N HCl$, factor preparations retained their original activity. It was also determined that the factor was dialyzable and was not extracted from aqueous solutions at either pH 7 or pH 1 by shaking with *n*-butanol or diethyl ether.

Attempts to chromatograph factor preparations on ion exchange resins not only resulted in extensive purification of the active principle, but also revealed more of its chemical properties. It was found that the factor was adsorbed on Dowex 2 chloride from active boiled extract preparations at neutral pH and subsequently was eluted with dilute HCl ($0.02 N$ to $0.1 N$). Under similar conditions, ATP and ADP remained adsorbed on the resin; 5-AMP was eluted earlier than the active factor. In $0.05 N HCl$, the factor was adsorbed weakly to Dowex 50 (hydrogen form) and could be eluted by further washing of the resin with $0.05 N HCl$. Under similar conditions, 5-AMP was not eluted from the resin, and ADP and ATP were eluted

considerably before the factor. By adsorption and elution on ion exchange resins, it has been possible to purify the factor by about 500-fold over the

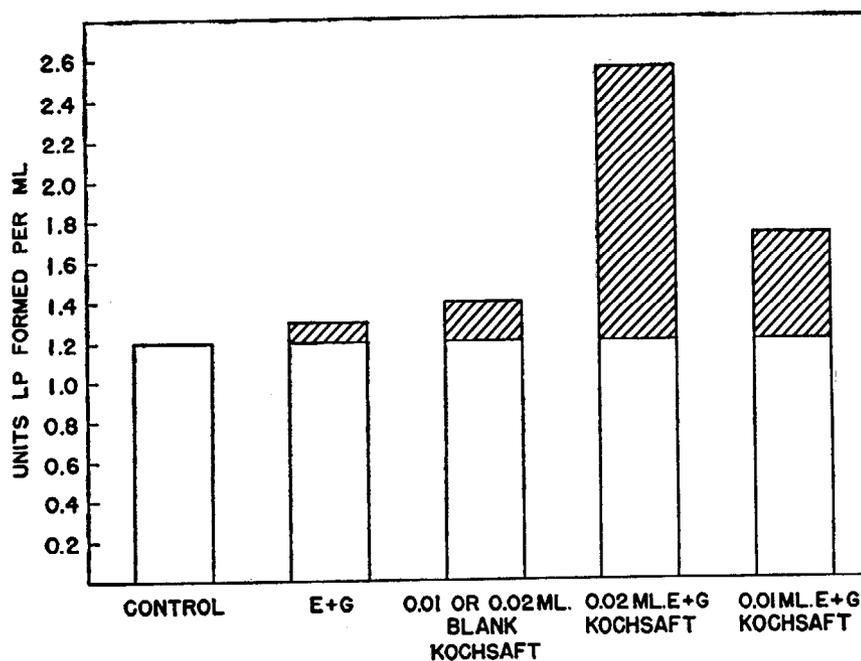


FIG. 4. The effect of preparations of the boiled extract on the formation of LP. Two 25 ml. portions of a suspension of washed particles in $0.25 M$ sucrose, derived from about 25 gm. of cat liver slices, were incubated with $2 \times 10^{-2} M$ Tris buffer (pH 7.4), $2.5 \times 10^{-3} M$ $MgSO_4$, and $1.7 \times 10^{-3} M$ ATP in a final volume of 30 ml. One vessel contained 150 γ of both *l*-epinephrine and glucagon (E + G), while the other contained no added hormones (blank). After shaking for 5 minutes at 30° , the flasks were heated in boiling water for 3 minutes and then chilled to 0° . The flask contents were centrifuged at $15,000 \times g$ for 15 minutes in the cold. 0.02 ml. and 0.01 ml. aliquots of the supernatant fluids (boiled extracts) were incubated with 0.13 ml. of an $11,000 \times g$ supernatant fraction of a dog liver homogenate in $4 \times 10^{-2} M$ Tris buffer (pH 7.4), $2.5 \times 10^{-3} M$ $MgSO_4$, and $1.7 \times 10^{-3} M$ ATP. The final volume was 0.20 ml. Control experimental vessels contained either water or 1.2 γ of both *l*-epinephrine and glucagon in place of the boiled extracts. LP activity was assayed before and after 5 minutes incubation at 30° , and the bars represent the amount of LP formed during this incubation period.

boiled extract, as judged by the lowering of optical density at $258 m\mu$ in relation to activity in stimulating LP formation in the $11,000 \times g$ supernatant fraction.³ As yet, no consistent differences in properties have been

³The active factor recently has been purified to apparent homogeneity. From ultraviolet spectrum, the orcinol reaction, and total phosphate determination, the

noted between factor preparations derived from the incubation of liver particles with epinephrine and those derived from incubation with glucagon.

Action of Factor in Supernatant Fractions of Liver Homogenates—Factor preparations were active in increasing LP formation in supernatant fractions of liver homogenates obtained by centrifugation at either 1200 or $11,000 \times g$. The effect of factor preparations was also apparent in supernatant fractions obtained by centrifugation for 45 minutes at $100,000 \times g$,

TABLE II
*Effect of Factor Preparations on Formation of LP in
 $100,000 \times g$ Supernatant Fractions*

0.13 ml. portions of the fractions from a homogenate of dog liver were incubated with 3.6×10^{-2} M Tris buffer, pH 7.4, 2.3×10^{-3} M $MgSO_4$, 1.6×10^{-4} M ATP, dephospho-LP (5.5 units per ml.), and the additions listed below. The final volume was 0.22 ml. The blank and active factor preparations were corresponding fractions collected during ion exchange chromatography of boiled extracts derived from the incubation of washed liver particles in the absence and presence of epinephrine, respectively. The boiled extracts were prepared as described (Fig. 4). LP activity was determined before and after 5 minutes incubation at 30° .

Fraction of homogenate	Additions	LP formed, units per ml.	Δ , units per ml.
$11,000 \times g$ supernatant fraction	Water	0.94	
	0.6 γ glucagon + 0.6 γ epinephrine	1.00	+0.06
	Blank factor preparation	0.97	+0.03
	Active factor preparation	1.48	+0.54
$100,000 \times g$ supernatant fraction	Water	3.00	
	0.6 γ glucagon + 0.6 γ epinephrine	3.09	+0.09
	Blank factor preparation	2.93	-0.07
	Active factor preparation	3.68	+0.68
Dialyzed $100,000 \times g$ supernatant fraction	Water	3.00	
	0.6 γ glucagon + 0.6 γ epinephrine	3.07	+0.07
	Blank factor preparation	3.26	+0.26
	Active factor preparation	3.85	+0.85

either before or after dialysis. In Table II are recorded the results of an experiment in which LP formation in a $100,000 \times g$ supernatant fraction before and after dialysis was compared with that in the $11,000 \times g$ supernatant fraction from which the $100,000 \times g$ supernatant fractions were derived. It can be seen that the formation of LP in the three fractions of

active factor appeared to contain adenine, ribose, and phosphate in a ratio of 1:1:1. Neither inorganic phosphate formation nor diminution of activity resulted when the factor was incubated with various phosphatase preparations, including prostatic and intestinal phosphatase and Russell's viper venom. However, the activity of the factor was rapidly lost upon incubation with extracts from dog heart, liver, and brain.

homogenates was increased by the active factor preparation, and the net increase in LP formation was nearly the same in all three cases. Thus it appeared that the formed elements were not required in the action of the factor on LP formation, nor was any readily dialyzable component of the supernatant fraction necessary.

It is of interest to note that the removal of the formed elements of liver homogenates by centrifugation at $100,000 \times g$ greatly increased the formation of LP in the resulting supernatant fraction. In preliminary experiments, the precipitate collected by centrifugation of homogenates between $11,000 \times g$ and $100,000 \times g$ (presumably consisting primarily of microsomes and glycogen) was found to inhibit strongly the formation of LP from dephospho-LP catalyzed by preparations of partially purified liver phosphokinase.

DISCUSSION

Differential centrifugation and microscopic examination of fractions obtained by centrifugation have shown that intact cells are not necessary components in the response of liver phosphorylase concentration to sympathomimetic amines and glucagon. The participation of added dephosphophosphorylase in the activation process and the ability to demonstrate hormone effects in previously frozen homogenates were considered additional evidence that intact cells were not necessarily involved. The absolute and relative activities of the sympathomimetic amines and glucagon in homogenates, in liver slices, and in intact animals indicate that the phenomena observed in homogenates may be related to the physiological activity of these agents. Although the demonstration that epinephrine and glucagon stimulate the net formation of LP in cell-free preparations surmounts the problem of dealing with intact cells, the analysis of the mechanism of action of the hormones is still complex.

It has been shown that the response to the hormones occurs in two stages, each of which may be eventually broken down into several steps. In the first stage, some portion of the particulate fraction of liver homogenates produces a heat-stable, dialyzable factor in the presence of the hormones. The identity of the particulate fraction to date has not been revealed by simple differential centrifugation experiments. The active factor produced by the particles in the presence of the hormones has been purified considerably, and it seems reasonable that identification of the active factor will yield important clues to the process involved in its production and to the mechanism by which it acts. The problem of identification of this substance is complicated by the probability that its molar concentration is extremely small in biological preparations.

In the second stage, this factor somehow influences the reactivation or

inactivation reactions occurring in the soluble fractions of homogenates, resulting in an increase in the formation of LP. In liver homogenates, the reactivation process (conversion of dephospho-LP to LP) is opposed by the action of LP phosphatase, and also may be inhibited by various components of homogenates, including the microsomal fraction. To date, data have not been conclusive enough to distinguish between a stimulation of the reactivation process by the hormones via the active factor and an inhibition of the inactivation of LP. Preliminary experiments have not shown reproducible effects of factor preparations on either purified phosphokinase or LP phosphatase; it is possible that the factor may undergo metabolic alteration before participating in or affecting one of the two processes.

It has been shown that heart contains enzymes capable of catalyzing the interconversion of LP and dephospho-LP as well as the interconversion of the heart phosphorylases (11). Recent experiments have shown that factor preparations from either heart or liver increased the conversion of dephospho-LP to LP when this reaction was catalyzed by extracts of dog heart. This suggests that tissues other than liver may possess some or all of the components involved in the response of liver homogenates to epinephrine.⁴

SUMMARY

1. The formation of liver phosphorylase from dephosphophosphorylase in cell-free homogenates of dog and cat liver was increased markedly in the presence of either epinephrine or glucagon in low concentration.

2. The relative activities of sympathomimetic amines in homogenates were similar to those observed in liver slices and in the intact animal.

3. The response to the hormones in liver homogenates was separated into two phases: first, the formation of an active factor in particulate fractions in the presence of the hormones and, second, the stimulation by the factor of liver phosphorylase formation in supernatant fractions of homogenates in which the hormones themselves had no effect.

4. The active factor was heat-stable, dialyzable, and was purified considerably by chromatography on anion and cation exchange resins.

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⁴ Active factor prepared from muscle particles of dog heart behaved in a manner similar to that of the factor from liver when chromatographed on ion exchange resins. In addition, it has been possible to observe production of an active factor in particulate preparations from dog skeletal muscle in the presence of epinephrine.

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